

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
<p>The public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing the burden, to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.</p> <p>PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.</p>					
1. REPORT DATE (DD-MM-YYYY) 26/Jul/2001		2. REPORT TYPE THESIS		3. DATES COVERED (From - To)	
4. TITLE AND SUBTITLE THE EFFECTS OF JP-8 JET FUEL ON THE IMMUNE SYSTEM OF TANK ENTRY WORKERS				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) LT COL RHODES AUDRY G				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) UNIVERSITY OF CINCINNATI				8. PERFORMING ORGANIZATION REPORT NUMBER CI01-208	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) THE DEPARTMENT OF THE AIR FORCE AFIT/CIA, BLDG 125 2950 P STREET WPAFB OH 45433				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT Unlimited distribution In Accordance With AFI 35-205/AFIT Sup 1					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES 23	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER (Include area code)

20010904 049

THE EFFECTS OF JP-8 JET FUEL ON THE IMMUNE SYTEM OF TANK ENTRY
WORKERS

A thesis submitted to the

Division of Research and Advanced Studies
of the University of Cincinnati

in partial fulfillment of the
requirements for the degree of

MASTER OF SCIENCE

in the Department of Environmental Health
of the College of Medicine

2001

by

Audry Gayle Rhodes

B.S., Brescia College, 1983
M.D., University of Kentucky, 1987

Committee Co-Chairs: Grace J. Lemasters, Ph.D.
James E. Lockey, M.D., M.S.

Abstract

Jet fuel is a common occupational exposure among commercial and military maintenance workers. JP-8 jet fuel, a military formulation, has been found to have immunotoxic effects in mice but little data exists for humans. The aim of this cross-sectional study was to determine if the number of immune cells in the peripheral blood was altered among tank entry workers, a group which has been determined in previous studies to have the highest exposure to JP-8 in the U.S Air Force. A total of 123 volunteers (45 tank entry workers) from three Air Force bases participated in the study. After adjusting for a number of covariates, tank entry workers were found to have higher numbers of white blood cells ($p=0.01$), neutrophils ($p=0.05$), and monocytes ($p=0.02$) and no differences in the numbers of total lymphocytes, T-cells, T-helper cells, T-suppressor cells, Natural Killer cells, and B-cells when compared with a low exposure group. Tank entry workers did not show any clinical effects of the increased immune cell counts. Although there were no differences in the number of lymphocytes among study groups, further investigations are needed to evaluate the functional ability of these cells to produce lymphokines and cytokines and modulate the immune system.

Acknowledgements

It is with sincere gratitude that I wish to acknowledge the contributions of the following individuals without whose advice and expertise I would not have accomplished this thesis.

Grace K. Lemasters, Ph.D., Professor and Director, Division of Biostatistics and Epidemiology, University of Cincinnati

James E. Lockey, M.D., M.S., Professor and Director, Division of Occupational and Environmental Medicine, University of Cincinnati

Colonel Roger Gibson, DVM, MPH, Ph.D., Principle Investigator Epidemiologist, Brooks Air Force Base, Texas

Lieutenant Colonel James W. Smith, Ph.D., Clinical Investigation Laboratory, Travis Air Force Base, California

James H. Yiin, M.P.H, Division of Biostatistics and Epidemiology, University of Cincinnati

This study was funded in part from a grant (T42/CC51042-07) sponsored by the National Institute for Occupational Safety and Health, Education and Research Center, Pilot Research Program, at the University of Cincinnati, Cincinnati, OH.

Table of Contents

	Page
List of Tables	2
Introduction	3
Background	5
Methods	7
Population	7
Questionnaire	8
Exposure Groups	9
Lymphocyte Analysis	9
White Blood Cell Count and Differential Analysis	11
Statistical Analysis	11
Results	11
Demographics and Life Style Characteristics	11
Exposure Levels	13
Lymphocytes and Subpopulations	13
White Blood Cells and Differential Counts	13
Discussion	14
Limitations	21
Bibliography	22

List of Tables

Table 1. Demographic and Life Style Characteristics by Exposure Group

Table 2. Breathing Zone and Breath Test Naphthalene Levels by Exposure Group

Table 3. Lymphocyte Subpopulation Counts by Exposure Group

Table 4. Lymphocyte Subpopulation Percentages by Exposure Group

Table 5. White Blood Cell Counts with Differential Counts by Exposure Group

Table 6. White Blood Cell Differential Percentages by Exposure Group

Table 7. Normal Values for White Blood Cell Indices

Introduction

The worldwide consumption of jet fuel approaches 60 billion gallons annually.¹ According to the U.S. Department of Labor, Bureau of Labor Statistics, 1.3 million workers were exposed to jet fuels in 1992. The U.S. Department of Defense uses Jet Propellant fuel type eight (JP-8), one formulation of jet fuel, at a rate of 3.5 billion gallons yearly, of which, the Air Force is the largest consumer. JP-8 is the battlefield fuel for all U.S. military operations and is expected to be in use well beyond the year 2025.²

JP-8 is a kerosene based fuel similar to commercial aviation fuel Jet A and Jet A-1, but has military additives which include antioxidants, static inhibitors, corrosion inhibitors, fuel system icing inhibitors, lubrication improvers, biocides, and thermal stability improvers. In 1996, JP-8 replaced JP-4, which was a more volatile and explosive, gasoline based fuel containing significantly more benzene, a proven carcinogen.³

In the Air Force, persons having the highest exposure to JP-8 are tank entry personnel. These persons enter on-board aircraft fuel tanks to perform inspections and maintenance activities. They are exposed to residual fuel in the tanks and to fuel released from reticulated polyurethane foam. The foam is fitted in fuel tanks of various aircraft and serves to reduce the risk of explosion from electrical arcing, lightning strikes, and static electricity. Fuel tanks with foam are less likely to explode if struck by ballistics or involved in a crash as the foam prevents fuel sloshing and spraying in the event of tank rupture. Tank entry personnel work in groups of three. The entrant works within the confined space of the tank and wears a respirator. Two attendants work near the tank entry port fetching tools and handling the fuel impregnated foam, which is usually

stacked on the aircraft wing while maintenance is occurring within the tank. The two exterior attendants do not wear respirators and therefore may have inhalation exposure to jet fuel while working near the tank opening or handling foam. All three wear cotton clothing rather than impermeable garments, which may generate static electricity. Fuel left within tanks or released from foam during handling, is readily absorbed and deposited onto the skin creating a dermal exposure.¹

While the Occupational Safety and Health Administration (OSHA) has not developed a permissible exposure limit (PEL) for jet fuel, the Air Force has occupational exposure limits of 350 mg/m³, Time Weighted Average (TWA) over 8 hours and 1,800 mg/m³ for short term exposures over 15 minutes. Tank entry personnel handling foam have been found to have exposures as high as 1,304 mg/m³ for an 8-hour TWA and 10,295 mg/m³ for a 15 min short-term exposure.^{1,4}

After the introduction of JP-8, fuel handlers complained of objectionable odors, skin irritation, dizziness and the persistent taste of jet fuel long after exposure.⁴ Health outcome studies in humans of the effect of JP-8 are limited, but some effects have been reported. Genotoxic changes as evidenced by sister chromatid exchanges were noted in aircraft maintenance workers however, no male reproductive effects have been noted on male semen parameters.⁵ Neurological disorders and hearing loss were also noted in aircraft maintenance workers occupationally exposed to jet fuel.⁶ Other reports on Swedish workers exposed to jet fuel cite effects on the nervous system.^{7,8,9} Postural balance deficiencies were noted in workers exposed to chronic low-levels of jet fuel.¹⁰

A reference report, published in 1998, by the Center for Disease Control's Agency for Toxic Substances and Disease Registry, indicated the toxicities of jet fuel are

not well defined and data gaps exist in many areas.¹¹ The immune system was identified as one area where human health effects needed further study and was the purpose of this research project.

Background

The immune system is responsible for regulatory responses to infection, cancer, autoimmune disease, and allergens. The spleen, thymus, lymph nodes, bone marrow, blood and other organs have cells involved in the immune response. In the peripheral blood, immune cells are represented by white blood cells that consist of lymphocytes, neutrophils, basophils, eosinophils, and monocytes.

Lymphocytes have several subpopulations that can be delineated by cluster designation (CD). T-cells (CD3) and B-cells (CD19) orchestrate the entire immune response. T-cells consist of T-helper cells (CD4) and T-suppressor cells (CD8) that modulate cell-mediated immunity both directly and by the secretion of lymphokines and cytokines. Natural Killer (NK) cells (CD56) target cancer cells.¹²

Most immune system toxicology studies concerning the health effects of jet fuel have been undertaken in mice. Exposure to inhaled benzene (a component of jet fuel) at levels of 50 ppm to 200 ppm over 7 days and 14 days produced a decreased ratio and absolute number of T-cells and B-cells in the blood and spleen. The effect was dose dependent and resulted in a suppressed ability to form antibodies.¹³ Subpopulations of T-cells were not addressed in the above benzene study and jet fuel as a complex mixture was not evaluated.

The effects of short-term (one hour daily for seven days) exposure of mice to JP-8, by inhalation, were a dose response decrease in weights of the spleen and thymus, and

a reduction in T-cell subpopulations in the lymph nodes. A decrease in circulating immune cells at low (100-250 mg/m³) concentrations was noted whereas at medium (500-1,000 mg/m³) concentrations the number of cells increased. High (2,500 mg/m³) concentrations appeared to be toxic to peripheral blood immune cells. Total T-cells were noted to decrease significantly at doses as low as 250 mg/m³ in the peripheral blood but absolute number and ratio of T-helper and T-suppressor cells were not evaluated. Macrophage percentages were also noted to decrease by two-thirds at low and high concentrations compared to unexposed mice.¹⁴ Long-term effects of short term exposure to inhaled JP-8 were studied to 28 days post-exposure. The weights of the spleen and thymus, initially decreased, returned to normal, and finally increased. At the high exposure of 2,500 mg/m³, immune cell numbers in the peripheral blood were substantially decreased at 1, 7 and 21 days, but were not noted to be statistically different from unexposed controls at 14 and 28 days.¹⁵ Again the subpopulations of immune cells were not delineated.

In experiments with mice exposed to JP-8, by dermal absorption, impairment in the induction of contact sensitivity and the generation of delayed-type hypersensitivity was found when the mice were later challenged by antigens.¹⁶ Splenic T-cells were noted to have significantly decreased proliferation rates compared to positive controls when stimulated, indicating a reduction in the functional capacity of the immune system. The number of circulating immune cells in the peripheral blood was not determined.

In humans, few immunotoxicity studies have been reported on exposure to jet fuel. One of note was a pilot study of exposed and unexposed workers, during the conversion of JP-4 to JP-8.³ In this study by Olsen et al, differences in the hematopoietic

system were noted but no significant findings were found in the immune system. Mean corpuscular hemoglobin and mean corpuscular volume were significantly lower in the exposed group while immune cells (total white blood cell counts and differential counts) were not significantly different. The sample size was small however, (18 exposed and 18 unexposed) and the lymphocyte subpopulations were not studied.

Methods

This investigation was a cross-sectional study designed to evaluate the effects of military jet fuel on the human immune system. The aim of this study was to determine if changes in the number of white blood cells or constituent components could be detected in the peripheral blood that may indicate abnormalities within the immune system.

Particular attention was devoted to lymphocyte subpopulations (T-cells, T-helper cells, T-suppressor cells, NK cells, B-cells), as these were the focus of several animal studies.

This study was part of a larger U.S. Air Force research project (The Acute Effects of JP-8 Jet Fuel).⁴

Population

Three Air Force bases in the Southeastern United States with significant numbers of personnel performing tank entry work were identified. Volunteers were solicited among tank entry personnel and other low and unexposed base personnel. Each participant was provided an incentive of fifty dollars. Institutional Review Board approval was obtained from the Air Force and University of Cincinnati and informed consents were signed.

To be included in the study subjects had to be active duty military personnel with a minimum of nine months on their current base. Tank entry personnel had to have one

or more hours of tank entry twice a week for at least nine months (validated against shop records). Personnel in the low exposure group had to have minimal exposure to fuel or solvents in the course of their routine work.

Excluded were those using alcohol within 12 hours prior to entering the study, suffering an injury requiring medical attention within the last six months, having a history of cancer, cerebral vascular accident, diabetes, or seizures, on medical profile, pregnant, or using hypertension medication, steroids, antacids or other heartburn medication, diet pills or other stimulants, tranquilizers or muscle relaxants, antidepressants, psychotherapeutic medication or large doses of megavitamins containing antioxidants.

Of the 189 volunteers, 4 did not meet inclusion criteria and 24 were no-shows on the day of sample collection and testing. Blood samples were collected on 123 personnel. A complete blood count was performed on all 123 samples and flow cytometry on 115.

Questionnaire

Each participant completed a questionnaire to determine current and past medical history, age, race, gender, months in present job title, body mass index (BMI), tobacco use, alcohol use and mental exertion. BMI (weight divided by height²) was calculated from responses to height and weight inquiries. Tobacco use during the preceding six months was dichotomized into smokers and nonsmokers. Alcohol use was determined by multiplying the average number of reported drinks per day times the average number of days per week alcohol was reportedly consumed. Alcohol use was then categorized as none, light (<10 drinks), moderate (10-30 drinks) and heavy (>30 drinks). Respondents were asked to rate the level of mental exertion on the job on a scale of 1 (lowest) to 9

(highest). Responses were grouped into thirds and categorized as mild, moderate, and heavy mental exertion.

Exposure Groups

One of the primary investigators for the larger Air Force study, an epidemiologist, convened a group of co-investigators, which by consensus categorized exposure levels by job title. Tank entry personnel were categorized as the high exposure group. Personnel in non-fuels related job titles (e.g. mechanics and information managers) were categorized as the low exposure group.

Another investigator for the larger Air Force study collected passive and breath levels of naphthalene, a surrogate for JP-8, on all participants. Passive levels were obtained by a breathing zone air-sampling device using an aluminum cartridge containing Tenax to capture volatile organic compounds on participants performing four hours of job-specific tasks. A sampling kit containing a 75 ml glass bulb with caps attached to each end was used to collect pre and post-task breath samples. Approximately 30 minutes preceding and 30 minutes following a four-hour task workers removed the caps and forcibly exhaled into the bulb and replaced the caps. Assays on the collected samples were performed by gas chromatography. The purpose was to determine an internal dose that would account for inhalation, dermal absorption and ingestion. The results of these assays were used to validate the categorization of exposure groups by job title.

Lymphocyte Analysis

To control for diurnal variation blood samples were collected in ten milliliter heparinized tubes during the post exposure phase of data collection, all within a two-hour window in the early afternoon. T-helper cells have been known to vary by 50% or more

depending on the time of day of collection.¹⁷ The specimens were packaged and sent overnight express under room temperature to the Travis Air Base Clinical Investigation Laboratory in California. Specimens were analyzed on arrival with the length of time from collection to analysis averaging 24 (range 22 – 26) hours.

Specimens were analyzed by flow cytometry. Becton Dickinson Immunocytometry Systems TRUCOUNT™ tubes containing a known quantity of beads were used to determine absolute counts of leukocytes. Fifty microliters of heparinized whole blood were added to two tubes, one containing 20 microliters of antibodies to CD3/CD8/CD45/CD4 and the other 20 microliters of CD3/CD16+CD56/CD45/CD19 antibodies. The tubes were capped, gently vortexed for 5 seconds and incubated for 15 minutes in the dark at room temperature. The tubes were uncapped and 450 microliters of FACS Lysing Solution was added to lyse red blood cells. The tubes were recapped, vortexed for 5 seconds and incubated for 15 minutes in the dark. Samples were then run on a Becton Dickinson Fluorescence-Activated Cell Sorter (FACSCalibur™ flow cytometer) using the MultiSET system and the Lyse/No-Wash technique. The flow cytometer was equipped to detect three-color fluorescence, forward scatter, and side scatter to determine the absolute count of lymphocytes and subpopulations (T-cells, T-helper cells, T-suppressor cells, Natural Killer cells, and B cells). The percent of these cells to total lymphocytes was calculated and the absolute number and percent of T-helper cells and T-suppressor cells comprising the T-cells population was also determined.

White Blood Cell Count and Differential Analysis

Three milliliters of blood was collected in a separate tube for a complete blood count (CBC). The CBC was processed by Coulter counter at each base's local clinical laboratory on the day of collection. The white blood cell count and machine generated differential were determined.

Statistical Analysis

The Fisher's Exact test was used to test statistical associations between exposure groups and categorical covariates. The mean, standard deviation, and range were determined for all outcome variables and continuous covariates. To determine significant differences in the means of the high and low exposure groups a normality test was performed on all outcome variables and those with a normal distribution were evaluated with a student t test and those that were non-normal or represented by percentages by a Wilcoxon rank sum test. Pearson Correlation analyses and Analysis of Variance (ANOVA) were applied to identify confounders that were significantly associated with both outcome variables and exposure levels. An Analysis of Covariance (ANOCOVA) using a general linear model procedure with backward elimination was employed to test the differences in outcome variables between exposure levels, while adjusting for other significant covariates. The SAS system was used for all analyses.

Results

Demographics and Life Style Characteristics

Table 1 lists the demographic characteristics by exposure group. There were 1.7 low exposure subjects for each high exposure enrollee. Differences were noted for tobacco use, race, age, gender and BMI. A disproportionate number of smokers were

Table 1. Demographic and Life Style Characteristics by Exposure Group

Covariates	White Blood Cell Analysis (n=123)	
	High Exposure	Low Exposure
	n (%)	n (%)
Subjects	45 (36)	78 (64)
Tobacco		
Smoker	21 (50)	21 (50)
Nonsmoker	21 (27)	57 (73)
P value	0.02	
Alcohol		
None	18 (44)	23 (56)
Light	5 (28)	13 (72)
Moderate	18 (31)	41 (69)
Heavy	1 (50)	1 (50)
P value	0.41	
Race (includes Hispanic)		
Caucasian	39 (43)	51 (57)
African American	2 (11)	17 (89)
Asian/Pacific Islander	1 (50)	1 (50)
Other	3 (25)	9 (75)
P value	0.02	
Hispanic*		
Hispanic	4 (33)	8 (67)
P value	1.0	
Gender		
Male	43 (40)	65 (60)
Female	2 (13)	13 (87)
P value	0.05	
Mental Exertion		
Mild	16 (32)	34 (68)
Moderate	21 (37)	36 (63)
Heavy	6 (46)	7 (54)
P value	0.61	
Age	Years	Years
Mean	23 .87	27.14
Std dev	4.30	6.17
Range	18.37	19.44
P value	0.002	
Months on job	Months	Months
Mean	47.20	50.04
Std dev	44.11	49.20
Range	7-172	1-223
P value	0.75	
Body Mass Index (BMI)	BMI	BMI
Mean	24.61	25.79
Std dev	3.24	2.87
Range	18.31	19-33
P value	0.04	

*Hispanic represents responses to 'also Hispanic' among respondents to questions regarding race.

P values for tobacco, alcohol, race, gender, Hispanic, and mental exertion calculated using Fisher's Exact test. P values for age, month on job, and BMI calculated with student t test.

Percent is composition of high vs low exposure group, rows add to 100%.

Significant ($p \leq 0.05$) levels are highlighted in bold.

noted in the high exposure group. African Americans and Hispanics were more common in the low exposure group. Only two females were represented in the high exposure group, a function of few females being employed as tank entry workers. The high exposure group was significantly younger than the low exposure group, although everyone in the study was less than 45 years old. BMI was higher in the low exposure group probably reflecting the older age of the participants. Alcohol consumption, mental exertion and months performing within the current job title did not differ among exposure groups. Therefore, on the variables that the two groups differ, an adjustment was performed in analysis.

Exposure Levels

Both passive Industrial Hygiene (IH) measures and post-task breath analysis of naphthalene differ significantly between exposure groups, which validate the characterization of exposure levels by job title (Table 2). The pre-task baseline breath analysis shows no significant difference between exposure groups indicating that high and low exposure personnel had a similar baseline exposure prior to performing their job on the day of testing.

Lymphocytes and Subpopulations

Flow cytometry results from 22 blood samples had to be discarded due to quality control issues (Lyse Wash protocol was used instead of Lyse No-Wash) leaving a total of 93 samples for statistical analysis. Tables 3 and 4 list results for the lymphocyte analysis. No significant differences were noted between high and low exposure groups.

White Blood Cells and Differential Counts

All samples were adequate for evaluation. White blood cells and differential

Table 2. Breathing Zone and Breath Test Naphthalene Levels by Exposure Group

	Micrograms/m ³ (n=123)	
	High Exposure	Low Exposure
Subjects	(n=45)	(n=78)
Industrial Hygiene		
Mean	583.23	2.47
Std dev	268.89	1.73
Range	123-1000	0.67-8.8
P value	<0.0001	
Pre-Breath		
Mean	0.75	0.71
Std dev	0.91	0.49
Range	0.33-6.1	0.33-2.8
P value	0.76	
Post-Breath		
Mean	3.80	0.80
Std dev	2.17	0.80
Range	0.9-11	0.33-6.9
P value	<0.0001	

Industrial Hygiene - breathing zone passive measures of naphthalene.

Pre-Breath - breath test measures of naphthalene pre-job exposure.

Post -Breath - breath test measures of naphthalene post-job exposure.

Significant (p<0.05) levels are highlighted in bold.

results are noted in Tables 5 and 6. Significant elevations of white blood cell counts (p=0.004), neutrophil counts (p=0.003), and monocyte counts (p=0.02) were noted in the high exposure group versus the low exposure group. After controlling for confounders (smoking and race) and other significant covariates (age, gender, and BMI), significant levels for these same outcome variables persisted; white blood cells (p=0.01), neutrophils (p=0.05) and monocytes (p=0.02).

Discussion

Though the primary Air Force study was related to the acute effects of jet fuel, this study was aimed at the potential health effects of chronic exposure. Tank entry workers

in the study were exposed to jet fuel twice a week for at least nine months prior to being studied.

Unlike previous animal studies, no effect on the peripheral blood T-cells was seen on flow cytometry analysis. It is difficult to compare jet fuel levels with naphthalene levels

Table 3. Lymphocyte Subpopulation Counts by Exposure Group

	Number of cells/mm ³ (n=93)	
	High Exposure	Low Exposure
Total lymphocytes		
Mean	2,041	2,065
Std dev	524	624
Range	1,019-3,245	962-3,658
P value	0.85	
T-cells		
Mean	1,520	1,509
Std dev	423	490
Range	632-2,574	655-2,866
P value	0.91	
T-sup cells		
Mean	550	545
Std dev	178	260
Range	179-1,050	196-1,633
P value	0.92	
T-help cells		
Mean	924	914
Std dev	283	284
Range	409-1,439	443-1,556
P value	0.87	
NK cells		
Mean	182	191
Std dev	96	95
Range	38-480	32-585
P value	0.68	
B-cells		
Mean	316	344
Std dev	119	166
Range	122-663	88-1,105
P value	0.39	

T-sup – T-suppressor cells, T-help – T-helper cells, NK – Natural Killer Cells

T-sup cells and T-help cells are subsets of T-cells.

P values calculated with student t test or Wilcoxon rank sum test

and correlate the inhalation exposures in this study but the experimental mice may have had comparable inhalation exposures to JP-8. It took inhalation exposures of 250mg/m³ (one hour for seven days) to decrease T-cell percentages in the animal's peripheral blood.¹⁴ The attendant worker falls short of this as other studies have reported 15 min STELs of 250mg/m³ and 8 hour TWAs of 200mg/m³.¹ The entrant worker wearing a

Table 4. Lymphocyte Subpopulation Percentages by Exposure Group

	Percent (n=93)	
	High Exposure	Low Exposure
T-cells		
Mean	74.19	72.88
Std dev	5.75	5.89
Range	61-84	50-84
P value	0.28	
T-sup		
Mean	27.22	26.09
Std dev	5.95	6.69
Range	15-39	15-46
P value	0.19	
T-help		
Mean	45.30	44.72
Std dev	6.58	5.65
Range	31-60	28-54
P value	0.87	
NK cells		
Mean	9.08	9.33
Std dev	4.43	3.67
Range	4-19	3-22
P value	0.44	
B-cells		
Mean	15.4	16.63
Std dev	4.2	5.45
Range	6-28	5-36
P value	0.29	

T sup – T-suppressor cells, T-help – T-helper cells, NK – Natural Killer Cells
T-cells, NK cells, and B-cells are percentages of Total Lymphocytes. T-sup and T-help cells are percentages of T-cells.

P values calculated with Wilcoxon rank sum test.

respirator would not be expected to have significant inhalation exposure.¹⁸ Dermal exposure appeared by observation to be substantial as the cotton clothes worn by tank entry workers and attendants were commonly drenched with JP-8. It has been estimated

Table 5. White Blood Cell Count with Differential Counts by Exposure Group

	Number of cells /mm ³ (n=123)	
	High Exposure	Low Exposure
White blood cells		
Mean	6,515	5,755
Std dev	1,402	1,309
Range	3,100-10,100	3,100-9,000
P value	0.004	
Neutrophils		
Mean	3,960	3,328
Std dev	1,267	1,030
Range	1,500-7,800	1,400-6,400
P value	0.003	
Lymphocytes		
Mean	1,827	1,799
Std dev	482	587
Range	1,000-3,200	100-3,700
P value	0.75	
Monocytes		
Mean	518	440
Std dev	193	155
Range	200-1,100	100-900
P value	0.02	
Eosinophils		
Mean	196	113
Std dev	165	125
Range	0-500	0-600
P value	0.18	
Basophils		
Mean	16	12
Std dev	37	32
Range	0-100	0-100
P value	0.53	

P values calculated with student t test or Wilcoxon rank sum test

Significant ($p \leq 0.05$) levels are highlighted in bold and represent levels of significance before adjusting for covariates.

that 100 mls absorbed and deposited on the skin of a 200 lb person is equivalent to the exposure level that produced immunotoxic effects in mice.¹⁶ Tank entry workers almost certainly experienced this level of exposure but no change in lymphocyte numbers were noted.

The complete blood count analysis done by Coulter counter showed increased white blood cell numbers, neutrophil counts and monocyte counts. The increase in white blood cells is a function of the increased neutrophils and monocytes. Neutrophils and monocytes are "professional" phagocytic cells.¹⁹ Neutrophils have a half life of about 6-20 hours in the peripheral blood and have the main task of ingesting bacteria although they are capable of binding and ingesting any appropriately opsonized material.²⁰ The neutrophil is a critical effector cell in humoral and innate immunity and plays vital roles in phagocytosis and bacterial killing.²¹ Monocytes in the circulating blood are transformed into macrophages in tissues, such as lung, liver, spleen, lymph nodes and skin. In the lung they are known as alveolar macrophages and in the skin as histiocytes and Langerhans cells. Macrophages can ingest solutes by pinocytosis and larger particles or microbes by phagocytosis.¹⁹ Macrophages and lymphocytes are the most significant cells of the immune system because of their release of lymphokines and cytokines that have wide ranging effects on host defense.

The reason for the elevations of neutrophils and monocytes is not clear. Smoking has been known to raise white blood cell counts but these effects persisted after adjusting for smoking. It cannot be explained by illness as exclusion criteria eliminated anyone with a significant medical condition or anyone ill on the day of testing. There have been reports of microbial (bacterial and fungal) colonization of jet fuel.²² An inhalation

exposure to bacteria, endotoxin, fungus or mycotoxin could possibly elevate the neutrophil and monocyte counts. The jet fuel would have to be aerosolized in order for that to happen, however. Exposure to vapors would not be sufficient. In observing tank entry workers involved in job specific tasks there were no grinding or blowing operations that would produce an aerosol. With intact skin, microbes should not enter the body to produce a systemic reaction.

Table 6. White Blood Cell Differential Percentages by Exposure Group

Subpopulations	Percent (n=123)	
	High Exposure	Low Exposure
Neutrophils		
Mean	59.65	57.16
Std dev	9.17	8.90
Range	39-82	33-79
P value	0.33	
Lymphocytes		
Mean	28.74	31.67
Std dev	7.06	8.13
Range	11-41	13-56
P value	0.11	
Monocytes		
Mean	8.06	7.76
Std dev	2.55	2.28
Range	2-14	2-13
P value	0.29	
Eosinophils		
Mean	3.06	2.93
Std dev	2.07	2.15
Range	0.5-11	0.5-11
P value	0.58	
Basophils		
Mean	0.49	0.48
Std dev	0.39	0.41
Range	0-1.7	0-1.9
P value	0.84	

P values calculated with Wilcoxon rank sum test.

It may be that jet fuel vapors create a systemic immune response unrelated to biological agents. An elevated white blood cell count has been observed in inhalation fever, a condition that can occur as the result of inhaling microorganisms but also by inhaling metal fumes, organic grain dust or pyrolysis products of fluoropolymers. The mechanism seems to be related to biochemical messengers mediating a systemic reaction.²³ In dermal exposure, fuel that is absorbed through the skin would almost certainly be ingested by Langerhans cells. These cells would proliferate and other macrophages would be recruited and if the fuel load were great the overall response would be increased numbers of monocytes entering the blood from the bone marrow. It is uncertain what the role of the neutrophil would be in a non-microbial foreign substance exposure.

It must be made clear that the elevation of white blood cells, neutrophils and monocytes in the exposure groups were almost entirely within the normal limits noted in

Table 7. Normal Values for White Blood Cell Indices*

	Number of cells/mm ³	
White Blood Cells	3,000-9,400	Percent of WBC
Neutrophils	1,000-6,400	40.2-75.4
Monocytes	200-800	4.2-12.6
Eosinophils	0-400	0-6.1
Basophils	0-100	0-1.3
Lymphocytes	800-2,800	14.9-45.8
		Percent of Lymphocytes
Natural Killer cells	90-590	5-27
B-cells	90-660	6-25
T-cells	690-2540	55-84
		Percent of T-cells
T-suppressor	190-1140	13-41
T-helper	410-1590	31-60

*Normal values are those cited by clinical laboratories performing analyses for this study.

Table 7. In reality, only four of the 124 enrolled subjects had elevated counts of one or more of these three lab tests. All four were in the high exposure group and were scattered among all three bases visited. These abnormalities were noted in individuals who, on the day of the study, denied present illness, significant medical history or medication use. One person had elevations of all three parameters that were also the highest levels seen in each category (WBC 10,200, neutrophils 7,800, and monocytes 1,100). These were not thought to be extreme enough to eliminate the subject from the study.

Limitations

Subjects were not randomly selected. Selection bias could have occurred with the use of volunteers. However it must be noted that almost all tank entry workers available for testing, volunteered, and were accepted into the study.

In cross-sectional studies, such as this one, associations can be drawn but causation cannot be determined. There was however significant control of covariates in the attempt to neutralize confounders.

Some of the subjects had to be eliminated from the lymphocyte analysis due to laboratory errors. This should have made little difference, as the percentage decrease in subjects was proportional among high and low exposure groups.

Immune cell counts can give an indication of the intactness of the immune system but does not measure the ability of these cells to function. Future studies should be directed at mitogen stimulation and proliferation assays that measure function and the ability to produce cytokines that regulate the immune system.

Bibliography

1. Carlton GN, Smith LB. Exposures to jet fuel and benzene during aircraft fuel tank repair in the U.S. Air Force. *Applied Occupational and Environmental Hygiene Journal*. 2000;15(6):455-91.
2. Survey of Jet Fuels Procured by Defense Energy Support Center, 1990-1996, report dated 9 Jun 1998. Defense Energy Support Center, Ft Belvoir, VA.
3. Olsen DM, Mattie DR, Gould WD, Witzmann F, Ledbetter M, Lemasters GK, Yiin J. A pilot study of occupational assessment of air force personnel exposure to jet fuel before and after conversion to JP-8. United States Air Force Research Laboratory, AFRL-HE-WP-TR-1998-0107, Sep 1998.
4. Smith LB. Risk assessment of acute exposure to jet fuel. Proposal # F-BR-200-0016-H, IERA/RSHI, Brooks AFB, TX, 3 Jan 2000.
5. Lemasters GK, Livingston GK, Lockey JE et al. Genotoxic changes after low-level solvent and fuel exposure on aircraft maintenance personnel. *Mutagenesis*. 1997;12(4): 237-243.
6. Kaufman L. A study of occupational neurological disorders and hearing loss in aircraft maintenance workers exposed to fuels and solvents. Dissertation in the Department of Environmental Health, College of Medicine, University of Cincinnati, 1998.
7. Knave B, Mindus P, Struwe G. Neurasthenic symptoms in workers occupationally exposed to jet fuel. *Acta Psychiatr Scand*. 1979;60(1):39-49.
8. Knave B, Olson BA, Elofsson S, et al. Long-term exposure to jet fuel. II. A cross-sectional epidemiologic investigation on occupationally exposed industrial workers with special reference to the nervous system. *Scand J Work Environ Health*. 1978;4(1):19-45.
9. Knave B, Persson HE, Goldberg JM, Westerholm P. Long-term exposure to jet fuel: an investigation on occupationally exposed workers with special reference to the nervous system. *Scand J Work Environ Health*. 1976;2(3):152-164.
10. Smith LB, Bhattacharya A, Lemasters GK, et al. Effect of chronic low-level exposure to jet fuel on postural balance of US Air Force personnel. *Journal of Occupational and Environmental Health*. 1997;39(7):623-632.
11. ATSDR. Toxicological profile for jet fuels JP-5 and JP-8. Agency for Toxic Substances and Disease Registry, CDC, Atlanta GA, 1998.
12. Salvaggio JE, Sullivan KA. The immune system and the environment. In: Rom WN, ed. *Environmental and Occupational Medicine*. Philadelphia: Lippincott-Raven; 1998:87-111.
13. Aoyama K. Effects of benzene inhalation on lymphocyte subpopulations and immune response in mice. *Toxicology and Applied Pharmacology*. 1996;85:92-101.
14. Harris DT, Sakiestewa D, Robledo RF, Witten M. Immunotoxicological effects of JP-8 jet fuel exposure. *Toxicology and Industrial Health*. 1997;13(1):43-55.
15. Harris DT, Sakiestewa D, Robledo RF, Witten M. Short-term exposure to JP-8 Jet fuel results in long-term immunotoxicity. *Toxicology and Industrial Health*. 1997; 13(5):559-570.

16. Ullrich SE. Dermal application of JP-8 jet fuel induces immune suppression. *Toxicological Sciences*. 1999;52:61-67.
17. Giorgi JV. Lymphocytes subset measurements: significance in clinical medicine. In: Rose N, Friedman H, Fahey J, eds. *Manual of Clinical Laboratory Immunology*, 3rd ed. Washington DC: American Society of Microbiology; 1986:236-246.
18. Pleil JD, Smith LB, Zelnick SD. Personal exposure to JP-8 jet fuel vapors and exhaust at air force bases. *Environmental Health Perspectives*. 2000;108(3):183-192.
19. Werb Z. Phagocytic cells: chemotaxis and effector functions of macrophages and granulocytes. In: Stites DP, Stobo JD, Fudenberg HH, Wells JV, eds. *Basic and Clinical Immunology*, 4th ed. Los Altos: Lange Medical Publications; 1982:109-123.
20. Douglas SD. Development and structure of cells in the immune system. In: Stites DP, Stobo JD, Fudenberg HH, Wells JV, eds. *Basic and Clinical Immunology*, 4th ed. Los Altos: Lange Medical Publications; 1982:87-108.
21. Burg ND, Pillinger MH. The neutrophil: function and regulation in innate and humoral immunity. *Clinical Immunology*. 2001;99(1):7-17.
22. Ferrari MD, Neirotti E, Albornoz C. Occurrence of heterotrophic bacteria and fungi in an aviation fuel handling system and its relationship with fuel fouling. *Rev Argent Microbiol*. 1998;30(3):105-114.
23. Rose CS, Blanc PD. Inhalation fever. In: Rom WN ed. *Environmental and Occupational Medicine*, 3rd ed, Philadelphia: Lippincott-Raven; 1998:87-111.